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Published in:
Poultry Science

DOI:
10.3382/ps.2012-02996

Fear, stress, and feather pecking in commercial white and brown laying hen parent-stock flocks and their relationships with production parameters

E. N. de Haas,*[1] B. Kemp,* J. E. Bolhuis,* T. Groothuis,† and T. B. Rodenburg‡

*Adaptation Physiology Group, Department of Animal Science, Wageningen University and Research, PO Box 338, 6700 AH, the Netherlands; †Behavioural Biology, Centre for Behaviour and Neuroscience, University of Groningen, NL Nijenborgh 7 9747 AG, the Netherlands; and ‡Behavioural Ecology Group, Department of Animal Sciences, Wageningen University and Research, PO Box 338, 6700 AH, the Netherlands

ABSTRACT Little is known about the relationship between welfare traits and production in laying hen parent stock (PS). In commercial laying hens and pure lines, it is known that aspects associated with reduced welfare such as high fear, stress, and feather pecking can have negative effects on production. Because PS hens are housed under different conditions than commercial laying hens, the relationship between welfare traits and production may differ. We therefore studied the fear response to a stationary person (SP) and novel object (NO), basal plasma corticosterone (CORT) and whole-blood serotonin levels (5-HT), and feather damage as a proxy for feather pecking in 10 Dekalb White (DW) and 10 ISA Brown (ISA) commercial PS flocks and related these to production data. Because the relationship between welfare traits and production may differ by genetic origin and group size, we also assessed genotype and group size effects. Dekalb White birds were more fearful of a SP, and had more feather damage and lower 5-HT levels than ISA birds. Genotypes did not differ in CORT. A large group size (n > 5,000) was associated with low feed intake and better feed conversion for ISA flocks. For DW flocks, high fear of the NO was associated with low BW, low egg weight, and low feed intake. For ISA flocks, high fear of the SP was associated with high mortality. For both lines, high CORT was related to low egg weight. This is the first study to associate levels of fear and CORT to production in commercial PS flocks. Management of PS flocks should take into account breed differences, group size effects, and effects of human-bird interactions. Further research is needed to determine the effects of fear, CORT, 5-HT, and feather damage in commercial PS flocks on the development of their offspring.

Key words: parent stock, laying hen, welfare, stress, productivity

2013 Poultry Science 92:2259–2269
http://dx.doi.org/10.3382/ps.2012-02996

INTRODUCTION

Parent-stock (**PS**) flocks produce eggs for production of commercial laying hens. To date, very little is known about the relationship between welfare traits and production in PS flocks. Coping with fear and stress and the development of feather pecking (**FP**) are aspects affecting the welfare of birds. In commercial laying hens—the offspring of PS flocks—numerous studies have shown that behavioral and physiological measurements related to welfare are associated with productivity (Shini et al., 2009; Sossidou and Elson, 2009; Sosnowka-Czajka et al., 2010; O'Connor et al., 2011; Nasr et al., 2012). For instance in laying hens, negative relationships have been found between fearfulness and egg production (Barnett et al., 1994; Uitdehaag et al., 2006; Uitdehaag et al., 2008a), FP and egg weight (Buitenhuis et al., 2004), FP and feed efficiency (Su et al., 2006), induced high basal plasma corticosterone (**CORT**) and oviposition time (Moudgal et al., 1991), and CORT and hen-day egg production in Japanese quail (Marin et al., 2002).

Parent-stock hens are hybrids of a 2-way cross of pure lines, housed in different conditions (floor housing) compared with pure lines (frequently cage-housed) and commercial laying hens (frequently housed in aviaries with or without outdoor range, at least in the Netherlands). Further, PS hens are housed together with roosters under a strict hygienic regimen that limits contact with humans. These factors may cause variation in how birds cope with fear and stress and the relationship between fear and stress with production may thus be different from what is known in pure lines and commercial laying hens. Moreover, it has been suggested that welfare levels are lower in PS birds in comparison with commercial hybrids due to higher levels of aggression and mortality (Sosnowka-Czajka et al., 2011).

Received December 20, 2012.
Accepted May 11, 2013.
[1]Corresponding author: elske.dehaas@wur.nl

Parent-stock hens produce fertilized eggs containing commercial laying hen embryos. Fear and stress in PS hens can affect the deposition of hormones in the egg (Henriksen et al., 2011), and these can, in turn, affect the developing offspring: the laying hen (Janczak et al., 2007; Guibert et al., 2011).

The level of fear and stress displayed by hens, and their predisposition to develop FP, can be related to their genetic origin. For example, commercial laying hens from a white genotype showed a longer duration of tonic immobility (antipredator response; Jones, 1996), indicating higher fearfulness, compared with various brown genotypes [ISA Brown (**ISA**), Colombian Blacktail, and Ixworth (Albentosa et al., 2003); brown Hyline hens (Fraisse and Cockrem, 2006)], Also, white commercial laying hens displayed greater CORT response to human handling than brown Hyline hens (Fraisse and Cockrem, 2006). In pure lines, hens from a White Leghorn origin (**WL**: white) were more fearful in various fear tests than hens from a Rhode Island Red origin (**RIR**: brown) and developed more feather damage due to FP than RIR hens when tested in conventional cages (Uitdehaag et al., 2008a). Purebred WL hens also had lower whole-blood serotonin (**5-HT**) levels than hens from a RIR origin (Uitdehaag et al., 2011). Lower 5-HT levels have been associated with high fearfulness and predisposition for FP (Bolhuis et al., 2009).

The relationship between behavioral and physiological measurements related to welfare and productivity has not yet been studied in PS flocks. Our aim was to assess the relationship between fear responses, physiological measurements of basal plasma-CORT and 5-HT, feather damage, and productivity in commercial PS flocks from a WL or RIR origin. Based on the literature mentioned above, our hypothesis was that flocks from a WL origin would be more fearful, and have higher levels of basal plasma CORT, lower levels of 5-HT, and higher levels of feather damage than flocks from a RIR origin. Additionally, we expected that high levels of fear, basal plasma CORT, and feather damage would be associated with reduced production. As group size varied between commercial flocks, we also assessed group size effects. Group size, under commercial conditions, is known to affect behavior and FP (Zimmerman et al., 2006).

MATERIALS AND METHODS

Birds and Housing

This study was approved by the Institutional Animal Care and Use Committee of Wageningen University. Two commercial PS genotypes were used: ISA and Dekalb White (**DW**). The ISA birds originate from a RIR origin, whereas the DW birds originate from a WL origin. The ISA flocks contained Rhode Island White hens with RIR (brown) roosters, whereas DW stock flocks contained WL (white) hens and WL (white) roosters. Rooster:hen ratio was 1:10 for all flocks. Ten ISA PS flocks and 10 DW PS flocks from Hatchery Ter Heerdt BV, Babberich, the Netherlands, were visited at 40 wk of age between August 2010 and August 2011. Flocks were housed on commercial propagator-farms, using floor housing with partly slatted floors. Houses provided a litter area and nest-boxes but no perches. Number of birds per meter squared was similar for all flocks (8 birds/m^2), but group size varied between 2,235 and 9,262 birds (DW: minimum = 3,941, maximum = 8,937; ISA: minimum = 2,235, maximum = 9,262). Light was provided for 15 to 16.5 h per day. We measured light intensities by means of a Voltcraft MS-1300 light meter (Conrad Electric Benelux, Oldenzaal, the Netherlands), measuring lux under a light source and not under a light source at 3 locations in the chicken house (front, middle, and back). Light intensities ranged from 1.3 to 42.1 lx (average 25.6 lx), with minimal daylight entering the house.

Production Parameters

During lay, production data of the flocks were recorded by the farmers from 20 until 65 wk of age. We used data that was consistently present for all flocks containing the average data from wk 25 until 40 (start), wk 40 only (top), and from wk 41 until 65 (end). For each production parameter per flock the following parameters were recorded:

- laying percentage (expressed as average number of eggs laid per day in relation to number of hens per flock),
- average egg weight (g) per flock based on weight of minimum 180 eggs per week,
- average feed intake per bird per day (g) based on feed intake per day divided by total number of birds per flock present,
- average feed conversion expressed as grams of feed/egg,
- average hen BW based on weight of 50 hens per week (only recorded during the start and the top period),
- cumulative mortality levels at the start and end (expressed as percentage of birds/flock that died), and
- occurrences of smothering events that led to mortality of large number of birds at specific time points (Bright and Johnson, 2011). We used farmers' reports to determine whether, at least once, a smothering event occurred in a particular flock.

Behavioral Parameters at wk 40 of Age

All behavioral observations were conducted at 40 wk of age, by 1 of 3 observers. Observation methods were brought into conformity with each other before the farm visits by comparing observations between observers. Number of samples and sample size per measurement were based on previous on-farm methodologies (e.g., Rodenburg et al., 2008) and based on guidelines of Welfare Quality (2009) to make reliable predictions for the whole flock.

Stationary Person Test

At 6 places in each chicken house, a stationary person (**SP**) test was performed, derived from the Welfare Quality protocol for on-farm assessment of welfare of poultry (2009). The observer, dressed in clothes similar to that of the farmer, walked through the chicken house at a slow pace (1 m per sec) and ceased walking on 6 predetermined locations: on the slats or litter area, always under a light source. The test locations were equally distributed over the chicken house (front, middle, and back). When the observer stopped walking, every 10 s, the number of birds within 25 cm of the observer was recorded for a total duration of 2 min. Proximity of 25 cm was based on the whole bird being within 25 cm of the SP. The latency until the first bird approached the SP and the average number of birds that approached the SP within the test duration were recorded. The average number of birds that approached was calculated by taking the average of all 12 time points. If no bird approached within the 2 min of the test, the maximum test duration was set at 130 s. The data from all 6 locations were averaged per flock. Whether any bird approached or did not approach within the 2 min of all the 6 tests was added to the data as a binary variable (yes/no). In 2 ISA flocks, no SP test was performed due to practical circumstances.

Novel Object Test

The procedure for the novel object (**NO**) test was comparable with the SP test, derived from the Welfare Quality protocol for on-farm assessment of poultry welfare (2009). On 6 different locations in the chicken house, the NO test was performed after the SP test had taken place (i.e., each SP test was followed by a NO test, but on a different location in the chicken house). A plastic stick (length: 50 cm, diameter: 3 cm) covered with colored tape markings (red, white, green, black, and yellow) was used as a NO. For all farms, the same NO was used. The NO was placed under a light source either on the slats or in the litter area with an equal distribution of places throughout the chicken house (front, middle, and back). After placing the NO, the observer withdrew to a distance of 2 m from the NO and recorded every 10 s, for a maximum duration of 2 min, the number of birds within 25 cm of the NO. We ruled out hens that approached the NO to escape the human observer, by placing the NO on the floor and then retracing our steps until we were 2 m distant from the NO. Hereby, we observed birds that came toward the NO and not away from the observer, but rather coming closer to the observer. The latency until at least 3 birds approached the NO and the average number of birds that approached were recorded. If no bird approached within the 2 min of the test, the maximum test duration was set at 130 s. The data from all 6 locations were averaged per flock. Whether any bird approached or did not approach the NO within the 2 min of all 6 tests was added to the data as a binary variable (yes/no).

Qualitative Behavioral Assessment

On 2 locations in the chicken house a qualitative behavioral assessment (**QBA**) was performed on flock level, based on the Welfare Quality protocol for on-farm assessment of welfare of poultry (2009). Scoring was done on a 6-point-scale of 20 behavioral expressions (e.g., distressed, fearful, relaxed, comfort, positively occupied; Welfare Quality, 2009). Low values indicate low levels of behavioral expression, whereas high values indicate high levels of behavioral expression. The output from both locations was averaged per flock, as no effect of location was found on QBA scores. Two ISA flocks were missing in the QBA assessment due to practical circumstances.

Feather Damage Score

Feather damage was assessed for 20 hens per flock at 40 wk of age. Hens were taken individually from the chicken house to an adjacent room. We randomized the location in the chicken house from where hens were taken (left, right and front, middle and floor, slats, and nest boxes). Choice of hen was based on the following principle: choose one hen and take the second closest to that hen. Choice of location where hens were taken from was alternated, and thus randomization of chosen hens was obtained. Feather damage to neck, back, and belly was assessed on a 3-point scale: intact/slight wear (a), moderate wear (b), and featherless areas (c), and summed to give a whole body index. The total score was 0 (all regions had "a"), 1 (only one "b" led to a total score of 1), or 2 (only one "c" led to a total score of 2; Welfare Quality, 2009). We calculated the average of 20 hens' feather damage scores per flock and the proportion of hens per flock with feather damage b or c per region (neck, back, and belly) to discriminate between regions for assessing different types of FP (Savory, 1995).

Blood Parameters at 40 wk of Age

Before feather damage scoring, 2.5 mL of blood was drawn from the wing vein of the hens (n = 20/flock). Flocks were sampled during the late morning (i.e., between 1000 and 1200 h), approximately 20 min after behavioral observations had taken place. Time of catching and postsampling was recorded to ensure samples were taken within 3 min. Blood was collected in 4-mL EDTA tubes and stored on ice immediately after blood collection.

Basal Plasma CORT

For basal plasma CORT analysis, 1.4 mL of blood was centrifuged at 2,095 × g at 21°C for 6 min to obtain plasma. Plasma was stored at −4°F (−20°C) before CORT was analyzed at the Department of Biomedical Sciences/Biochemistry at the University of Veterinary Medicine in Vienna (Austria). Plasma (0.5 mL) was extracted with 5 mL of diethyleter. After evaporation of the ether and redissolving steroids in assay buffer (0.5 mL), an aliquot (50 μL) was measured in a CORT enzyme immunoassay (described in detail by Palme and Mostl, 1997).

5-HT

For 5-HT analysis, 1.1 mL of blood was stored at −112°F (−80°C). Blood samples were thawed and serotonin concentration (nmol/mL) was assessed by fluorescence assay. Fluorescence was determined in a Perkin-Elmer 2000 Fluorescence spectrophotometer at 283 and 540 nm after blood samples were prepared; see description by Bolhuis et al. (2009).

Statistics

Data were analyzed with SAS 9.2 (SAS Institute Inc., Cary, NC). The experimental unit was flock. The GLM consisted of fixed effect of genotype (DW or ISA), and the linear effect of group size (to test for group size effects) and the interaction between genotype and group size for production parameters, CORT, 5-HT, and feather scoring. A backward regression procedure was applied in case group size or the interaction genotype × group size effect was below a P level of <0.05. Effects of genotype and group size (group size bigger or smaller than 5,000) on the occurrences of smothering were tested with a GenMod procedure with a binary distribution and a logit link function. The latency to approach in the SP and NO was not distributed normally due to a positive exponential distribution (i.e., a large number of data points at the far extreme). Therefore, we assessed effects of genotype and group size (bigger or smaller than 5,000) on the binary variable (approaching yes/no) by means of a GenMod procedure with binary distribution and logit link function. An exploratory factor analysis with orthogonal rotation was used to determine similarity of (normally distributed) variables in the QBA. Factors with an eigenvalue of >1 were retained in the analysis. For the effects of physiology and feather damage on production, we used a GLM with the fixed effect of genotype, and the linear effect of group size, to which (independently) average CORT, average 5-HT, or average feather damage were added as covariables, including their interaction with genotype. Due to lack of variation in behavioral response to the NO and SP in ISA and DW flocks, respectively, only within-genotype relationships with production could be assessed for either the NO (DW) or SP (ISA). For the effects of behavior in the NO and SP on production variables, we used a GLM with the linear effect of group size to which (independently) latency to approach and number of birds that approached were added. The relationship between production and NO was, thus, assessed within the DW genotype only, and the relationship with production and SP was assessed within the ISA genotype only. Only main effects and interactions with P-value <0.05 are presented.

RESULTS

Production Parameters

Table 1 shows the production parameters per genotype during the start, top, and end of the production period. In the top period, DW hens had a lower BW than ISA hens ($F_{1,17}$ = 13.1, P = 0.003). Egg weight was lower for DW hens in the end period ($F_{1,19}$ = 4.24, P = 0.05). During the start of the production period, DW birds had a lower feed conversion than ISA birds ($F_{1,19}$ = 8.3, P = 0.01). Feed conversion at the top and end of production was lower in large ISA flocks compared with small ISA flocks, whereas no group size effects were found for DW flocks (topβ_{ISA} −39 g: $F_{1,19}$ = 8.22, P = 0.01, endβ_{ISA} −17 g: $F_{1,19}$ = 4.85, P = 0.04). In small ISA flocks, mortality levels until wk 40 of age were higher than in large flocks, whereas in DW flocks mortality levels were unaffected by group size (genotype × group size: $F_{1,18}$ = 5.04, P = 0.04, small flocks with less than 5,000 birds: 5.1 vs. 3.9% for large flocks with more than 5,000 birds). The number of flocks in which smothering occurred was higher for ISA flocks than for DW flocks (X_2^1 = 13.1, P = 0.003, Table 1), and higher for small flocks than for large flocks (X_2^1 = 5.3, P = 0.02, probability of a smothering event occurring: 70% for large flocks vs. 0% for small flocks). Feed intake per bird per day was higher in small flocks compared with large flocks at the start of the production period ($F_{1,19}$ = 13.8, P = 0.002; small flocks: 129.4 vs. 124.0 g/bird per d, for large flocks). No other effects of genotype, group size, or their interaction on production parameters were found.

Behavioral Observations

In only 10% of the DW flocks, birds approached the SP before the test ended, whereas in 75% of the ISA flocks, birds approached before the end of the test (X_2^1 = 8.4, P = 0.007; Table 2). No effect of group size was found on response to the SP. Of all the flocks, irrespective of genotype, in which birds approached, the average latency for the first bird to approach was 78 ± 12 s, with on average 2.4 ± 0.5 birds approaching within the duration of the test. In only 30% of the ISA flocks, birds approached the NO, whereas in 90% of the DW flock, birds approached the NO (X_2^1 = 10.66,

Table 1. Average production parameters of Dekalb White (DW) and ISA Brown (ISA) parent-stock flocks on production parameters at the start (25–40 wk), top (wk 40), and end (41–65 wk) of the laying hen production period

Age	Production parameter	DW (n = 10)	ISA (n = 10)	*P*-value
Start: wk 25–40	Hens in lay (%)	93.9	91.9	0.06
	Egg weight (g)	58.2 ± 0.4	58.7 ± 0.4	0.12
	Hen BW (g)	1,662.0 ± 33	1,872.0 ± 26	0.24
	Cumulative mortality (%)	4.2	4.9	0.26
	Feed intake (g/bird per d)	116.0 ± 0.8	120.0 ± 0.8	0.33
	Feed conversion (g of feed/egg)	124.0 ± 1.0[a]	131.0 ± 1.7[b]	0.01
Top: wk 40	Hens in lay (%)	93.3	92	0.12
	Egg weight (g)	60.4 ± 0.8	60.8 ± 0.4	0.14
	Hen BW (g)	1,686.0 ± 22[a]	1,920.0 ± 18.3[b]	0.003
	Feed intake (g/bird per d)	122.0 ± 1.7	130.0 ± 3.0	0.12
	Feed conversion (g of feed/egg)	136.0 ± 2.3	149.0 ± 3.6	0.002
End: wk 41–65	Hens in lay (%)	88.9	88.7	0.19
	Egg weight (g)	60.3 ± 0.8[a]	61.6 ± 0.2[b]	0.05
	Cumulative mortality (%)	5.9[a]	7.8[b]	0.03
	Feed intake (g/bird per d)	118.6 ± 1.3	119.8 ± 1.2	0.39
	Feed conversion (g of feed/egg)	133.5 ± 2.1	135.1 ± 1.6	0.42
Overall: wk 25–65	Occurrences of smothering events (% of farms)	10[a]	80[b]	0.003

[a,b]Means with different superscripts between columns indicate differences ($P < 0.05$).

P = 0.001). In small flocks, the likelihood that birds approached the NO was smaller than in large flocks ($X_2{}^1 = 7.49$, $P = 0.006$, likelihood to approach 60% in small flocks vs. 80% in large flocks). Of all the flocks, irrespective of genotype, in which birds approached the NO, the latency for the first 3 birds to approach was 60.3 ± 10.2 s with on average 4.6 ± 1.14 birds approaching within the duration of the test.

Qualitative Behavioral Assessment

Exploratory factor analysis revealed 3 factors for the QBA (Table 3). Factors were labeled based on the expression that loaded most strongly on the factor, leading to factor 1 "distressed," factor 2 "comfort," factor 3 "active," explaining, respectively, 53, 25, and 7% of the variance. The DW flocks had higher scores for the factor "distressed" than ISA flocks ($F_{1,16} = 16.5$, $P = 0.002$), but no differences were found between the 2 genotypes for the factor "comfort" ($F_{1,16} = 0.16$, $P = 0.69$) or the factor "active" ($F_{1,16} = 0.40$, $P = 0.55$); Table 2. Group size or its interaction with genotype did not affect factor scores.

Feather Damage Score

Dekalb White hens had a higher average feather damage score than ISA hens ($F_{1,19} = 9.83$, $P = 0.006$, Table 2). This was caused by a higher proportion of DW hens/flock with belly damage ($F_{1,19} = 8.1$, $P = 0.02$) and back damage ($F_{1,19} = 7.0$, $P = 0.02$) in comparison with ISA flocks (Figure 1). Group size or its interaction with genotype did not affect feather damage score.

Blood Parameters

Basal plasma CORT did not differ between genotypes ($F_{1,19} = 0.09$, $P = 0.76$, Table 2). The 5-HT levels were higher in ISA hens than in DW hens ($F_{1,19} = 10.0$, $P = 0.005$). Neither group size nor the interaction with genotype affected the blood parameters.

Table 2. Fear response, feather damage, and physiological data of Dekalb White (DW) and ISA Brown (ISA) parent-stock flocks at 40 wk of age

Test variable	DW	ISA	*P*-value
Stationary person test			
Number of flocks in which hens approached (expressed as % of the total flocks)	10[a]	75[b]	0.007
Novel object test			
Number of flocks in which hens approached (expressed as % of the total flocks)	90[a]	30[b]	0.001
Qualitative behavioral assessment			
Factor 1 "distressed"	0.6 ± 0.3[a]	−0.8 ± 0.2[b]	0.002
Factor 2 "comfort"	−0.08 ± 0.2	0.1 ± 0.5	0.69
Factor 3 "active"	0.1 ± 0.4	−0.2 ± 0.3	0.55
Feather damage score			
Average feather damage score (0–2)	1.0 ± 0.1[a]	0.5 ± 0.1[b]	0.006
Physiological measurement			
Basal plasma corticosterone (ng/mL)	1.05 ± 0.1	0.99 ± 0.1	0.76
Whole blood serotonin (nmol/mL)	53.0 ± 5.3[a]	79.1 ± 6.4[b]	0.005

[a,b]Means with different superscripts between columns indicate differences ($P < 0.05$).

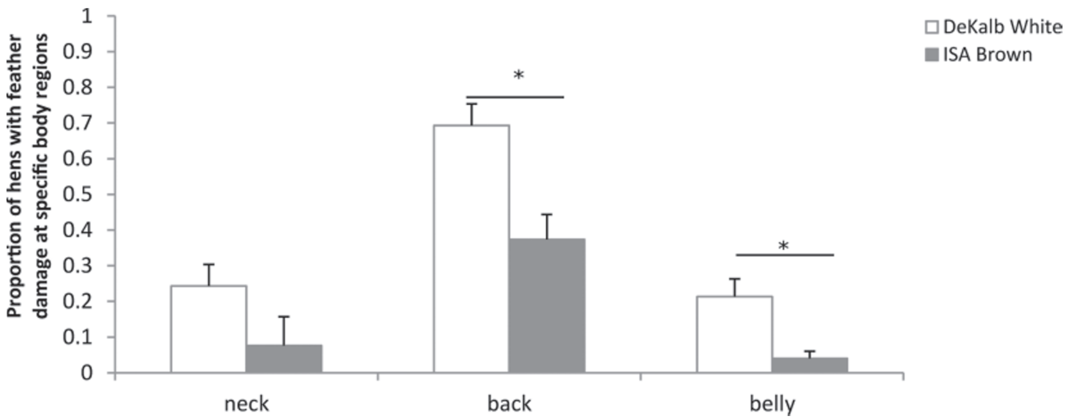

Figure 1. Proportion of 20 hens per flock with feather damage on the neck, back, and belly region in Dekalb White and ISA Brown parent-stock laying hen flocks at 40 wk of age. $^*P = 0.02$.

Relationship Between Production Parameters and Fear Response, QBA, Feather Damage, and Physiological Measurements

The relationships between fear response, QBA, feather damage, physiological measurements, and production parameters are given in Table 4.

Fear Behavior and Production

Due to lack of variation in the behavioral response to the NO and SP in ISA and DW flocks, respectively, only within-genotype relationships with production could be assessed for the NO (DW only) or SP (ISA only). Only when including the ISA flocks that approached, a long latency to approach the SP and a low number of birds that approached the SP was related to high mortality levels at the end of production (latency: β +0.05%, $F_{1,5} = 7.70$, $P = 0.05$, number of birds that approached: β −1.12%, $F_{1,5} = 7.78$, $P = 0.05$). Only when including the DW flocks that approached, a long latency to approach the NO was related to low egg weight at the top (β −0.03 g, $F_{1,8} = 9.53$, $P = 0.02$) and at the end of production (β −0.06 g, $F_{1,8} = 15.19$, $P = 0.01$). A long latency to approach the NO was also related to a low BW of DW hens at the top of production (β −1.49 g, $F_{1,8} = 9.11$, $P = 0.02$). If more DW hens approached the NO, the feed intake per bird was higher at the end of production (β +0.70 g/d, $F_{1,8} = 2.40$, $P = 0.05$).

Qualitative Behavioral Assessment and Production

Over genotypes, flocks which had high scores for the QBA factor “comfort” had hens with a higher BW at the top of production than flocks with low scores for this factor (β +2.6 g: $F_{1,15} = 4.95$, $P = 0.05$). Of the DW flocks that had high scores for the QBA factor, “comfort” also had higher egg weight at the top period ($\beta_{\text{Dekalb White}}$ +2.51 g: genotype × group size interaction: $F_{1,16} = 7.52$, $P = 0.02$). Flocks with a high score for the QBA factor “active” had lower feed intake per bird at the top and end of production than flocks with a low score (β_{top} −5.7 g/d: $F_{1,16} = 6.4$, $P = 0.03$ and β_{end} −2.3 g/d: $F_{1,16} = 6.8$, $P = 0.03$). The QBA factor “distressed” was not related to any of the production parameters.

Physiology and Production

High basal plasma CORT was related to low egg weight at the top (β_{top} −1.55 g: $F_{1,19} = 11.4$, $P = 0.01$) and at the end of production (β_{end} −0.47 g: $F_{1,19} = 8.8$, $P = 0.01$, Figure 2 and Table 4). High levels of feather damage in wk 40 were related to lower mortality levels during the start of production (β −2.7%: $F_{1,18} = 7.89$, $P = 0.02$).

Table 3. Factors based on an exploratory factor analysis of behavioral expressions of the qualitative behavioral assessment

Item	Factor 1 Distressed	Factor 2 Comfort	Factor 3 Active
Distressed	0.9	−0.2	−0.02
Fearful	0.9	−0.4	0.2
Scared	0.9	−0.3	−0.2
Tense	0.9	−0.3	−0.2
Unsure	0.9	−0.3	−0.2
Nervous	0.9	−0.3	−0.2
Frustrated	0.9	0.1	−0.3
Bored	0.9	0.1	−0.4
Depressed	0.8	0.2	−0.4
Agitated	0.7	−0.4	−0.2
Comfort	−0.4	0.9	−0.1
Calm	0.2	0.9	−0.2
Positively occupied	0.04	0.9	0.2
Content	−0.2	0.9	0.1
Happy	−0.3	0.8	−0.2
Relaxed	−0.3	0.8	−0.14
Friendly	−0.4	0.7	−0.3
Confident	−0.6	0.6	−0.2
Active	−0.3	−0.1	0.9
Energetic	−0.4	−0.1	0.8

Table 4. Linear effects [positive (+) or negative (−) β-values] of fear response, qualitative behavioral assessment (QBA) factors, feather damage, and physiological measurements on production parameters of Dekalb White (DW) and ISA Brown (ISA) parent stock flocks[1]

	Production parameter				
Item	Hen BW (g)	Egg weight (g)	Cumulative mortality (%)	Feed intake (g/bird per d)	Feed conversion (g of feed/egg)
Stationary person test (ISA only)					
Latency to approach (s)	$P > 0.05$	$P > 0.05$	$+0.05^{end}$	$P > 0.05$	$P > 0.05$
Number of hens that approached	$P > 0.05$	$P > 0.05$	-1.12^{end}	$P > 0.05$	$P > 0.05$
Novel object test (DW only)					
Latency to approach (s)	-1.49^{top}	-0.03^{top}; -0.06^{end}	$P > 0.05$	$P > 0.05$	$P > 0.05$
Number of hens that approached	$P > 0.05$	$P > 0.05$	$P > 0.05$	$+0.7^{end}$	$P > 0.05$
QBA					
Factor 1: Distressed	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
Factor 2: Comfort	$+2.6^{top}$	$+2.51_{DW}{}^{top}$	$P > 0.05$	$P > 0.05$	$P > 0.05$
Factor 3: Active	$P > 0.05$	$P > 0.05$	$P > 0.05$	-5.7^{top}; -2.3^{end}	$P > 0.05$
Feather damage					
Average feather damage/flock	$P > 0.05$	$P > 0.05$	-2.7^{start}	$P > 0.05$	$P > 0.05$
Physiological measurement					
Plasma corticosterone (ng/mL)	$P > 0.05$	-1.55^{top}; -0.47^{end}	$P > 0.05$	$P > 0.05$	$P > 0.05$
Whole-blood serotonin (nmol/mL)	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

[1]QBA with 20 behavioral expressions (Welfare Quality, 2009). Factors 1, 2, and 3 were respective factors based on an explanatory factor analysis of the 20 QBA behavioral expressions; $P > 0.05$ = P-value > 0.05; ^{start} = start of production between 25 and 40 wk of age; ^{top} = top of production at 40 wk of age; ^{end} = end of production: between 40 and 65 wk of age.

DISCUSSION

Although much research has been done on laying hens, not much is known about their parents. Such knowledge might help to understand their behavior, stress sensitivity, and variation in production parameters. We analyzed genotype differences and group size effects on production, behavior, feather damage, and physiology as well as the relationship among these. For production performance standards for PS hens of DW, see http://www.isapoultry.com/en/Products/Dekalb/Dekalb%20White.aspx, and for PS hens of ISA, see http://www.isapoultry.com/en/Products/Isa/Isa%20Brown.aspx.

Production

Dekalb White PS hens had a lower BW, a lower feed conversion, and produced lighter eggs compared with ISA PS hens. This is in line with other studies in purebred layer lines and commercial lines (Benyi et al., 2006; Singh et al., 2009; Bonekamp et al., 2010; Silversides, 2010). The differences in BW and feed efficiency between DW and ISA hens are most probably due to genetic differences in body constitution and activity patterns between hens derived from a white line and brown line (Luiting, 1990). In small flocks, mortality was higher in ISA flocks than in DW flocks. Hens from a brown line often have higher mortality than hens from

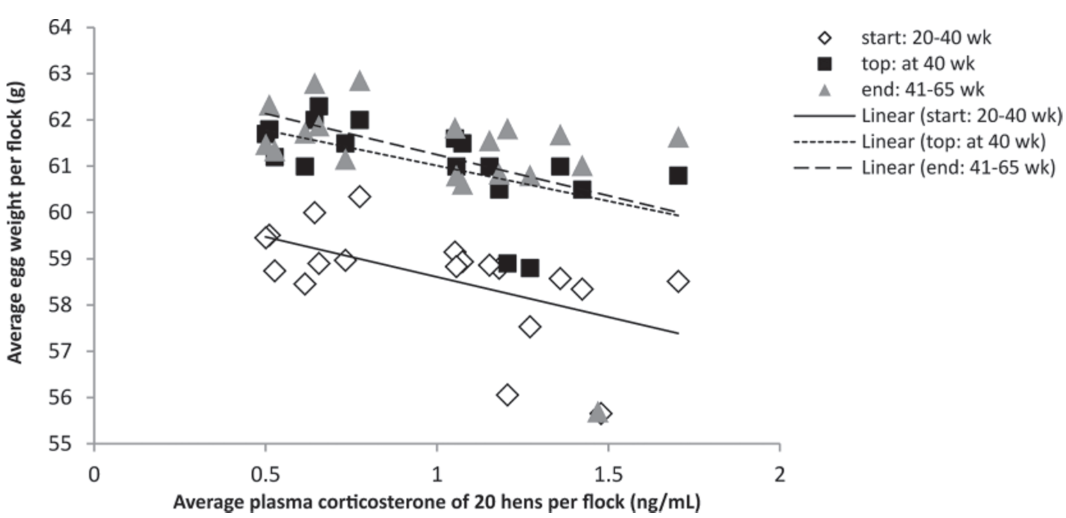

Figure 2. Relation between average plasma corticosterone and egg weight in parent-stock laying hens during the start, top, and end of production.

a white line when reared in conventional cages (Singh et al., 2009), housed at point-of-lay in furnished cages (Wall et al., 2008), or housed in floor systems (Tauson and Abrahamsson, 1996). We also found higher occurrences of smothering in ISA flocks than in DW flocks. It is known by the industry (see management guide for PS: http://www.isapoultry.com) that hens from brown lines have a stronger tendency to crowd than hens from white lines, which can cause mortality due to smothering. Smothering has been associated with panic and hysteria (Mills and Faure, 1990). In ISA hens, social adherence seem to also play a role (http://www.isapoultry.com). Smothering in or near the nests mostly occurs during onset and peak of lay (Bright and Johnson, 2011), which we also noticed (data not shown). Other forms of smothering can be observed in the litter area or even in the outdoor run (Sparks et al., 2008; Rodenburg et al., 2012). Smothering occurred most frequently in small flocks. Because density was the same for all flocks, small flocks were housed in barns with a smaller surface than large flocks, providing less space for escaping smothering events. Because DW birds have 13% lower BW than ISA birds, the effective stocking density of ISA birds under the same number of hens per meter squared of DW birds may constrain the actual space per hen and thereby increase the risk of smothering in ISA flocks. The ISA birds also had higher feed conversion and higher mortality in small flocks, which was not the case for DW birds. These results indicate that DW birds were able to cope with varying group sizes, whereas ISA birds appeared to perform better in large flocks. This is in line with findings that hens from a brown line showed more behavioral problems in smaller flocks than in larger flocks (Zimmerman et al., 2006).

For both genotypes, feed intake per bird per day was lower in large flocks than in small flocks. Because there was no effect of group size on BW, this could be related to the increase in activity in large flocks. This is supported by the negative correlation between the QBA factor activity and feed intake. In large flocks there may be more competition for food, which leads to increased activity. This in turn may lead to birds eating more often, and probably in smaller portions.

Behavior, Physiology, and Feather Damage

Partially confirming our hypothesis, we found that DW birds in our study showed higher fear of a SP, had higher scores for QBA factor "distressed," and had lower 5-HT levels than ISA birds. Hens from a white line are known to be more fearful in various fear tests than hens from a brown line (Mahboub et al., 2004; Fraisse and Cockrem, 2006; Uitdehaag et al., 2009). Higher fear of humans in DW birds fits with previous findings that cage housed pure WL hens were more fearful than RIR hens when approached by a human holding a NO (Fraisse and Cockrem, 2006; Uitdehaag et al., 2008a,b). In contrast, DW birds were less fearful of the NO than ISA birds. In the previously mentioned tests (Mahboub et al., 2004; Fraisse and Cockrem, 2006; Uitdehaag et al., 2009), hens from a white line showed higher fear in tests with human involvement than hens from a brown line. It appears that hens from a white line do not necessarily have higher general fear levels than hens from a brown line, but show higher fear of humans specifically. The finding that the loadings for the QBA factor "distressed" were higher for DW than for ISA flocks also indicate that DW flocks appeared more distressed to the observer than ISA flocks. In summary, DW flocks appear to be more fearful toward humans, which opens the route for improvement of the human-bird relationship.

Unexpectedly, DW and ISA hens did not differ in basal plasma CORT levels. For both genotypes, CORT represented basal levels (<1.5 ng/mL; Cockrem, 2007). White commercial layers differ from brown layers in CORT response but not in basal levels, which could be similar for the PS hens in our study (Fraisse and Cockrem, 2006). Whole-blood serotonin was lower in DW hens than in ISA hens. Pure cage-housed WL hens also had lower whole-blood 5-HT than cage-housed RIR hens (Uitdehaag et al., 2011). Activity of the brain serotonergic system shows an inverse relationship with FP (van Hierden et al., 2002, 2004). Hens from a low FP line (Buitenhuis et al., 2006) and a line selected for low mortality (mainly due to reduced FP and cannibalism; Bolhuis et al., 2009) also had higher blood 5-HT levels compared with their counterparts. Hens from a white line frequently have more feather damage than hens from a brown line (Uitdehaag et al., 2006; Biscarini et al., 2010). Our results fit with previous studies both for 5-HT and for feather damage as the DW hens had more feather damage than the ISA hens. Feather damage in PS hens is partly caused by mating, especially damage to the back. However, the higher proportion of hens with feather damage to the belly region in DW flocks points to the presence of vent pecking (Savory, 1995). The prevention of vent pecking may thus require extra attention in white flocks, which is addressed in the management guide for PS management of ISA (http://www.isapoulty.com).

Relationships Between Production, Behavior, Physiology, and Feather Damage

Our expectations were that high fear, CORT, and feather damage would negatively affect production. Indeed, in DW flocks, a long latency to approach the NO was associated with a low egg weight and a low BW. Also, in flocks where many hens approached the NO, the birds feed intake per day was high. Taken together, these results indicate that high levels of fear in a flock may have caused a poorer production performance. As high levels of fear can affect the stress response (de Haas et al., 2012), this may negatively affect production performance (Barnett et al., 1992). In a genetic association study, associations between fear (measured by duration of tonic immobility) and egg weight were found as well as associations between the response to a NO and growth in male offspring of a WL and red jungle-fowl cross (Schütz et al., 2004). A freezing response to a NO has also been associated with reduced hen-day egg-production and tendencies were found for a reduced BW (Uitdehaag et al., 2008a). High fear of a NO could affect egg weight and BW by coinciding selection for the same loci. Conversely, if low fear of the NO (high number of birds approaching) relates to high feed intake, this can positively affect BW and egg weight. Also, flocks which scored high on the QBA factor "comfort" had a higher hen BW, and in the DW flocks also higher egg weight. Within and between lines, flocks which were less fearful (relationship between response to NO and BW in DW flocks) and appeared more comfortable (both lines), generally had higher BW than more fearful and less comfortable flocks.

In ISA flocks, a long latency to approach the SP and a low number of hens approaching the SP was associated with high mortality levels at the end of production. Fear of humans has been associated with production traits in laying hens (Barnett et al., 1992), but not with mortality. However, in non-beak-trimmed hens selected for low mortality, escape attempts waned sooner after a human suddenly appeared in front of their cage than in hens that were not selected for low mortality (Bolhuis et al., 2009). A cause of high mortality within ISA flocks was due to smothering events. Although the cause of these events was unclear, a combination of avoidance of the farmer out of high general fear levels (i.e., seen by high fear of the NO) and social adherence with various underlying causes could have caused smothering. Also for the ISA flocks, the human-bird relationship should be taken into account to reduce the risk of high mortality by reducing fearfulness.

High levels of feather damage were related to low mortality at the start of production. Feather damage can originate from FP, which can lead to mortality due to cannibalism (Savory, 1995). A possible explanation for the unexpected negative relationship between feather damage and mortality is that birds with severe damage or cannibalized birds have been taken out of the flocks before we measured feather damage at 40 wk. What remains in the flocks are hens with low levels of feather damage in flocks with initial high mortality due to FP but with a lower density. In PS flocks, however, feather damage and associated effects should be further investigated, as the damaging effect roosters have on hens' feather damage cannot be excluded.

We also found that high basal plasma CORT was related to low egg weight at the top and end of production. Elevated CORT is associated with enhanced energy expenditure, due to increased protein and lipid metabolism in avian species (Pilo et al., 1985; Shini et al., 2009). In relation to protein metabolism, high CORT leads to increased net breakdown of protein especially in muscle tissue in broilers (Lin et al., 2006; Mumma et al., 2006; Dong et al., 2007). There are 2 possible explanations for the relationship between CORT levels and egg weight. First, albumen and yolk weight are reduced by lack of available protein due to enhanced proteolysis caused by high CORT. Albumen comprises 60% of the egg weight and consists mainly of proteins (Moran, 1987). As high CORT intensifies proteolysis, a trade-off for albumen synthesis may take place, causing a reduction in egg weight. For yolk (33% of egg weight), treatment with CORT in zebra finches was shown to inhibit yolk precursor production (Salvante and Williams, 2003), which consequently affects yolk synthesis (Moran, 1987). Second, retention time of the egg in the oviduct may be shortened due to high CORT. Hens injected with CORT after ovulation had a shorter interval from injection to oviposition than hens injected with progesterone (Etches and Cunningham, 1976). Time in the oviduct affects eggshell weight, which comprises 12% of the egg weight (Melek et al., 1973). A shorter time in the oviduct may thus restrict eggshell development, but also formation of albumen and yolk, which are dependent on protein synthesis in the oviduct (Moran, 1987). Thus egg formation may be penalized due to high basal CORT levels. High basal CORT may be high due to high fear levels, and thus possibly indicative of chronic stress, which should be reduced to minimize negative effects on production and welfare of PS flocks. Additionally, because egg weight and maternal CORT levels can also affect offspring quality, including stress sensitivity (Henriksen et al., 2011), further research is needed to determine the effect of fear, stress, and feather damage in commercial PS flocks on the development of their offspring.

In summary, this is the first study to show that fear, stress, and feather damage is associated with production in PS laying hens. The DW flocks were generally more fearful of humans than ISA birds. Low levels of fear of a NO were associated with higher BW and egg weight in DW birds. In ISA birds, increased fear of humans was associated with higher mortality. Our study also showed that ISA birds were more at risk to smothering events than DW birds, especially in small flocks, possibly due to social adherence either due to fearful events or other causes. Management of PS flocks should therefore take the human-bird relationship into account and realize that measures can have differential effects for ISA and DW hens in large and small flocks.

ACKNOWLEDGMENTS

We acknowledge Ter Heerdt BV hatchery (Babberich, the Netherlands) and its farmers for their collaboration. Gratefully acknowledged are the following: Ger de Vries-Reilingh of the Adaptation Physiology Group for her technical assistance on farm and in the laboratory; Rudie Koopmanschap (Adaptation Physiology Group, Wageningen UR, the Netherlands), Courtney Daigle (Michigan State University, East Lansing), and Merel Verhoeven (Livestock Research Centre, Wageningen UR, the Netherlands) for their assistance in the 5-HT assay; and Sophie Rettenbacher and Rupert

Palme of the Department of Biomedical Sciences/Biochemistry at the University of Veterinary Medicine in Vienna (Austria) for the basal plasma CORT analysis. Panagiotis Sakkas (Animal Nutrition Group, Wageningen UR, the Netherlands) is gratefully acknowledged for his contribution to the discussion of the manuscript. This study was supported by the Division of Earth and Life Sciences with financial aid from the Netherlands Organization for Scientific Research and the Ministry of Economic Affairs, Agriculture and Innovation within the program "The Value of Animal Welfare."

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